

Ameliorative Effects of Monofloral *Cistus Creticus* Bee Pollen on the Oxidant-Antioxidant Systems in Streptozotocin-Induced Diabetic Rats

Sibel Taş^{1,*}, Aycan Tosunoğlu^{1,2}, Melahat Dirican^{1,3}, Cansu Nur Tekin^{1,4} & Emre Sarandöl^{1,5}

¹Bursa Uludağ University, Sciences and Arts Faculty, Department of Biology, 16059, Bursa,

*Corresponding author: smeral@uludag.edu.tr

²Türkiye. aycanbilisik@uludag.edu.tr

³Türkiye. mdirican@uludag.edu.tr

⁴Türkiye. cansunurkoksas@gmail.com

⁵Türkiye. sarandole@uludag.edu.tr

Received: (7/10/2023), Accepted: (19/11/2023), Published: (1/9/2024).

DOI: [10.59049/2790-0231.1215](https://doi.org/10.59049/2790-0231.1215)

ABSTRACT

This study investigated the influence of *Cistus creticus* bee pollen (CCP) on oxidative stress and metabolic changes in streptozotocin (STZ)-induced diabetic rats. Twenty-eight healthy male Wistar rats were split into four groups: control (C), CCP-treated C (C+CCP), diabetes (D), and CCP-treated D (D+CCP) groups. To induce diabetes in rats, a single dose intraperitoneal injection of STZ (65 mg/kg) was administered. CCP (350 mg/kg/day) was applied to the drinking water of the rats for four weeks after diabetes was established. Serum glucose, insulin, total cholesterol (TC), triglyceride (TG), and high-density lipoprotein-cholesterol (HDL-C) levels were evaluated by an autoanalyzer. Malondialdehyde (MDA) levels in both plasma and tissues (heart, kidney, liver, and skeletal muscle) were by spectrophotometric method. Commercial kits were used to detect serum paraoxonase (PON), arylesterase (ARE), superoxide dismutase (SOD), and blood glutathione peroxidase (GSH-Px) enzyme activities. In the D+CCP group, serum glucose, TC, and TG levels decreased, and insulin levels increased significantly ($p < 0.05$). In the groups designated as C+CCP and D+CCP, the study revealed a significant decrease in malondialdehyde (MDA) levels in plasma, kidney, liver, and muscle tissues, alongside a significant increase in serum paraoxonase (PON) and arylesterase (ARE) enzyme activities ($p < 0.05$). These findings support our hypothesis that CCP strengthens the antioxidant system and improves oxidative stress and metabolic chaos in diabetes. In conclusion, our study suggests a potential benefit of CCP as a therapeutic and/or adjunctive agent that improves diabetes mellitus and its related complications.

Keyword: Diabetes mellitus; oxidative stress; hyperlipidemia; *Cistus creticus*; bee pollen.

INTRODUCTION

Bee pollen is a raw material that honeybees transport to the hive for feeding larvae by collecting from flowering plants and mixing bee secretions and nectar. Since ancient times, it has been used as a food supplement as well as to treat or prevent many diseases [1]. Bee pollen contains proteins, carbohydrates, lipids, fatty acids, amino acids, minerals, vitamins, and bioactive molecules, such as flavonoids and phenolic compounds [2, 3]. Honeybees usually bring pollen granules to the hive from various and most efficient pollen sources, depending on the floral structure of the region. Therefore, pollen

granules of a wide variety of plants are transported to the hive at the same time, and commercially, they are often marketed as mixtures. For this reason, the use of mixtures of pollen granules belonging to different plant species in research makes it impossible to work with standard material. Standard quality pollen with minimal variations, obtained by collecting bee pollen from a single plant taxon, is termed monofloral pollen [4]. Monofloral bee pollen has the potential to be a reliable alternative or supplement in medicinal practice [5] and for this reason, research is being done to distinguish pollen granules according to their types and to bring

influence on metabolic alterations, including serum levels of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), insulin, and blood glucose. Additionally, it aims to investigate the effects of CCP on oxidative status through the assessment of malondialdehyde (MDA) levels in plasma and various tissues (heart, muscle, liver, and kidney). Furthermore, it aims to study the antioxidant status of CCP in rats by measuring serum paraoxonase (PON), arylesterase (ARE), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) enzyme activities.

Materials and methods

Palynological purity analysis

To acquire pollen loads, we used many colonies of *Apis mellifera* Anatolia placed in Langstroth-type hives in the Bursa/Türkiye lowland area. We collected pollen loads in the heavy pollen season of the *Cistus creticus* flowers. We removed the accumulated pollen loads from the bottom pollen drawers and kept in at -20°C until the application. To test the amount of *C. creticus* pollen in the samples taken, 10 parallel samples were prepared from the whole mix by homogenizing pollen pellets with 70% ethyl alcohol. Slides prepared by using the Wodehouse [32] method and unacetolysed pollen grains were examined by light microscopy to determine the purity of the samples. A thousand pollen grains were identified randomly from each of the slides and CCP purity was calculated as 99,96 %.

Experimental design

Twenty-eight healthy male Wistar rats with an average weight of 350 g were obtained from the Bursa Uludağ University animal house. Four rats were housed in each cage and standard humidity (55±5%), temperature (25±2°C), and light/dark cycle (12/12 hours) conditions were kept. The rats were divided into four groups (n:7); control rats (C), control rats given CCP (C+CCP), diabetic rats (D), and diabetic rats given CCP (D+CCP).

Induction of diabetes

To induce diabetes, streptozotocin (STZ) (Sigma-Aldrich Chemicals, USA) was dissolved in citrate buffer (pH 4.5) and a single dose of 65 mg/kg was administered

intraperitoneally to D and D+CCP groups. For rats, blood glucose levels above 200 mg/dL were well-considered diabetic and used for the experimental process. Blood glucose values were determined by a glucometer (Abbott, USA) in a drop of blood cut from the tail end of the rats once a week.

Cistus creticus pollen treatment

Seven days after injection of STZ, CCP (350 mg/kg/day) was introduced into the drinking water of the groups C+CCP and D+CCP for 30 days. Drinking water containing CCP was prepared daily. Daily food and water consumption of all experimental groups were followed and body weights were measured once a week.

Sample collection and estimation of biochemical parameters

Thirty days after the experimental procedure, blood samples were drawn via cardiac puncture under light ether anesthesia. The blood was then centrifuged at 3000 rpm for 15 minutes to obtain the blood supernatant. Lipid profile, insulin, and antioxidant enzyme analyses were conducted on the blood supernatant. Blood samples were collected into tubes containing EDTA for MDA (Malondialdehyde) analysis.

Plasma and serum samples were stored at -20°C until they were analyzed. After the blood draw, tissues from the gastrocnemius muscle, heart, kidney, and liver were dissected for further evaluation. The dissected tissues were rinsed with cold standard saline solution and then stored at -20°C for MDA analysis.

Serum total cholesterol (TC), triglycerides (TG), and HDL-cholesterol (HDL-C) levels were measured using an Architect C8000 autoanalyzer (Abbott Laboratories, IL, USA). Superoxide dismutase (SOD) and glutathione (GSH) enzyme activities, as well as serum insulin levels, were determined using ELISA kits from Bioassay Technology Laboratory, China. Serum paraoxonase (PON) and arylesterase (ARE) enzyme activities were measured with commercial kits from Rel Assay Diagnostics, Turkey, using the Architect C8000 device. Tissue MDA levels were studied following the method defined by Ohkawa *et al.* [33] and the results were

Plasma and tissue MDA levels were found to be significantly increased in group D than in group C. Plasma and tissue MDA

levels (except the muscle in C+CCP) were significantly decreased in the C+CCP and D+CCP groups compared to those of the C and D groups, respectively.

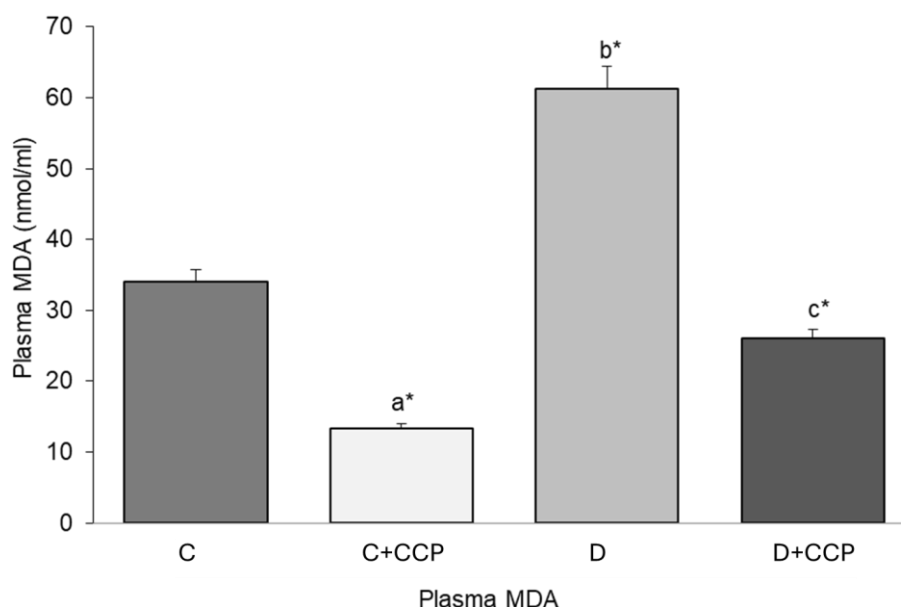


Figure (1): Plasma malondialdehyde (MDA).

Values are given as mean±SEM (standard error of the mean) Statistical comparison: ^aC vs C+CCP, ^bC vs D, ^cD vs D+CCP. Statistical

significance, * $p < 0.05$ for abbreviations of study groups, see Table 1.

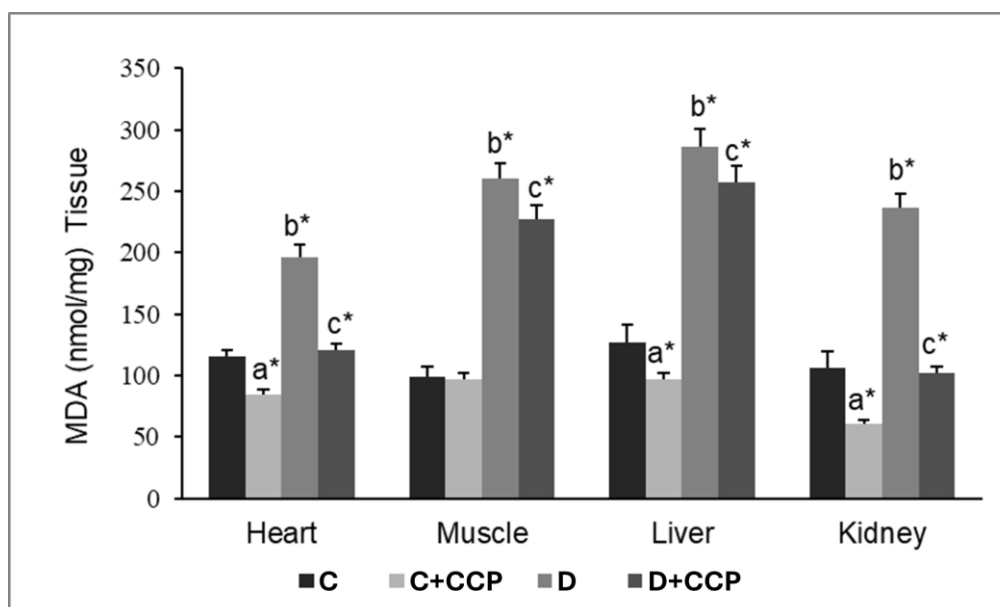


Figure (2): Tissue malondialdehyde (MDA) levels. Values are given as mean±SEM (standard error of the mean). Statistical comparison: ^aC vs C+CCP, ^bC vs D, ^cD vs D+CCP. Statistical significance, * $p < 0.05$, for abbreviations of study groups, see Table 1.

DISCUSSION

In this study, the increase in plasma and tissue MDA levels and changes in antioxidant enzyme activities demonstrated oxidative stress in STZ-induced diabetic rats. In addition, increases in blood glucose, serum TC and TG levels indicate metabolic deterioration due to diabetes [15, 35].

Hypoglycemic effect

The decrease in blood glucose levels accompanied by the increase in insulin levels in the D+CCP group may indicate that the pancreatic tissue destroyed by STZ is regenerated with the use of CCP. These results support the findings of previous studies that polyphenols and flavonoids can regenerate pancreatic tissue in diabetes [36]. In addition, other reasons for the diminished blood glucose level in the D+CCP group may be that pollen inhibits the activities of digestive enzymes that are effective in carbohydrate digestion, and/or the pollen affects glucose transporters, as reported in various publications [19-21]. Another remarkable feature of pollens is their antihyperlipidemic [30, 37] effects, and it is thought that this means diabetic patients can be protected from diabetes-related atherosclerotic cardiovascular complications [38, 39].

Hypolipidemic effect

Just as observed in prior research, this study also identified elevated serum TG levels in diabetic rats. However, the administration of pollen treatment was found to ameliorate hypertriglyceridemia. The increase in TG levels and its improvement with treatment may be associated with the decreased insulin levels in diabetes which was increased with CCP treatment. As it is known, when insulin levels decrease plasma TG levels increase since hormone-sensitive lipase activity cannot be inhibited. Lipid-lowering drugs and drugs used to treat diabetes reduce the number of events related to cardiovascular complications, but many patients seek natural alternatives for treatment because drugs can have side effects [40, 41]. When the improvement in blood glucose and lipid levels, observed in this study, is taken into account, CCP can be considered a good alternative for supportive treatment.

Oxidative stress-reducing effect

Our hypothesis that CCP has an antioxidant effect like other pollens has been proven by the significant decrease in plasma and tissue MDA levels in the D+CCP group compared to the D group. Since lipids are the substrate for lipid peroxidation, decreased TC and TG levels, observed in the present study, can be assumed one of the factors contributing to the decrease in MDA levels [42].

In this study, an increase was detected in serum SOD, and GSH-PX enzyme activities, and a decrease in plasma PON, ARE, and enzyme activities in diabetic rats. SOD and GSH-PX fight together to neutralize the free radicals that are released. The increased SOD and GSH-PX activities, observed in the D group, might be a protective response against the oxidative stress observed in the present study [43]. Compared with the D group, there were not any significant changes in SOD and GSH-PX activities in the D+CCP group.

PON is an antiatherogenic HDL-bound enzyme that protects LDL against ROS and hence oxidative damage. As in this and other studies, a reduction in PON and ARE enzyme activity has been found in many conditions such as diabetes, hyperglycemia, hyperlipidemia, and oxidative stress. Abbott *et al.* [44] stated that a difference in the binding of paraoxonase to HDL may occur because of an abnormality in the structure of HDL in diabetic conditions and a conformational change in paraoxonase may also occur. Another reason for the decreased PON activity observed in group D may be the decrease in enzyme expression due to oxidative damage. The significant decrease in ARE activity observed in group D can be considered an indirect indicator of the decrease in paraoxonase enzyme production because in some studies it has been suggested that ARE activity reflects the enzyme mass [44, 45]. Pollen has a rich content of flavonoids and it has been shown in studies that flavonoid-rich components increase the activity of the PON enzyme [30, 46]. One of the important findings of this study is that CCP increases PON and ARE enzyme activity in C+CCP and D+CCP groups. The increase in both PON and ARE enzyme activities in this study may have occurred due to the

improvement in oxidative stress related to the antihyperglycemic, antihyperlipidemic, and antioxidant effects of CCP. However, the increased PON and ARE activities observed in the C+CCP group may also indicate a direct antioxidant effect of CCP. In addition, the decreased plasma MDA levels observed in the C+CCP group may be another clue to the direct antioxidant effect of the pollen.

The CCP exhibits potential effects in lowering blood sugar levels, reducing lipid levels, and mitigating oxidative stress, which may introduce confounding factors like diet and exercise that could have influenced the outcomes.

The limitations of our study are clinical and preclinical investigations, which are needed to approve our study findings and to recognize the CCP pharmacokinetic and dynamic efficacy in addition to the phytochemical screenings, which are necessary to identify the molecules in CCP responsible for their hypoglycemic, hypolipidemic, and oxidative stress-reducing effects.

CONCLUSION

The current study findings suggest a potential benefit of CCP as an antihyperglycemic, antihyperlipidemic, and antioxidant agent and may be a promising adjunctive agent in the treatment of diabetes and/or reducing diabetes-related complications. However, since there is no previous study of monofloral CCP in diabetes, we propose the need for additional research to illustrate the therapeutic and supportive potential, as well as to uncover the underlying mechanisms responsible for its beneficial impacts in diabetes and other conditions linked to metabolic disruptions.

Authors' contributions

Sibel Taş, Ayca Tosunoğlu, Emre Sarandöl and Melehat Dirican designed and conducted the study, including data collection, data analysis and interpretation. Cansu Nur Tekin collected some of the manuscript data. Ayca Tosunoğlu worked on the concept of the study. Sibel Taş, Ayca Tosunoğlu and Emre Sarandöl prepared the manuscript. All authors approved the final manuscript.

Ethical approval

All experimental procedures involving animal use adhered to approved ethical policies and procedures. (Bursa Uludag University, Ethics approval number: 2019-04/08).

Consent for publication

All the authors have read and approved the manuscript for publication

Competing interest

The authors declare that there is no conflict of interest.

Acknowledgement

This research received no grant from any funding agency/sector.

REFERENCES

- 1] Denisow B, Denisow-Pietrzyk M. Biological and therapeutic properties of bee pollen: A review. *Journal of the Science of Food and Agriculture*. 2016;96(13):4303-9.
- 2] Sawicki T, Starowicz M, Kłębukowska L, Hanus P. The profile of polyphenolic compounds, contents of total phenolics and flavonoids, and antioxidant and antimicrobial properties of bee products. *Molecules*. 2022;27(4):1301.
- 3] Komosinska-Vassev K, Olczyk P, Kaźmierczak J, Mencner L, Olczyk K. Bee pollen: chemical composition and therapeutic application. *Evidence-Based Complementary and Alternative Medicine*. 2015;2015.
- 4] Ketkar SS, Rathore AS, Lohidasan S, Rao L, Paradkar AR, Mahadik KR. Investigation of the nutraceutical potential of monofloral Indian mustard bee pollen. *Journal of Integrative Medicine*. 2014;12(4):379-89.
- 5] Spulber R, Doğaroğlu M, Băbeanu N, Popa O. Physicochemical characteristics of fresh bee pollen from different botanical origins. *Romanian Biotechnol Lett*. 2018;23:13357-65.
- 6] Reisner W, Gartlehner K. Entwicklung einer maschinen-tauglichen Identifikationsmethode für Blütenpollen.

- of different botanic origins. *Italian Journal of Food Science*. 2015;27(2):120-31.
- 25] Papaefthimiou D, Papanikolaou A, Falara V, Givanoudi S, Kostas S, Kanellis AK. Genus *Cistus*: a model for exploring labdane-type diterpenes' biosynthesis and a natural source of high value products with biological, aromatic, and pharmacological properties. *Frontiers in chemistry*. 2014;2:35.
- 26] Huang H, Shen Z, Geng Q, Wu Z, Shi P, Miao X. Protective effect of *Schisandra chinensis* bee pollen extract on liver and kidney injury induced by cisplatin in rats. *Biomedicine & Pharmacotherapy*. 2017;95:1765-76.
- 27] Premratanachai P, Chanchao C. Review of the anticancer activities of bee products. *Asian Pacific journal of tropical biomedicine*. 2014;4(5):337-44.
- 28] Atsalakis E, Chinou I, Makropoulou M, Karabournioti S, Graikou K. Evaluation of phenolic compounds in *Cistus creticus* bee pollen from Greece. Antioxidant and antimicrobial properties. *Natural Product Communications*. 2017;12(11):1934578X1701201141.
- 29] El-Seedi HR, Eid N, Abd El-Wahed AA, Rateb ME, Afifi HS, Algethami AF, et al. Honey bee products: Preclinical and clinical studies of their anti-inflammatory and immunomodulatory properties. *Frontiers in Nutrition*. 2022;8:761267.
- 30] Taş S, Sarandöl E, Tekin CN, Tosunoğlu A, Vatan Ö, Hürriyet H, et al. Monofloral *brassica nigra* pollen improves oxidative stress and metabolic parameters in streptozotocin-induced diabetic rats. *Journal of Applied Biological Sciences*. 2022;16(3):563-74.
- 31] Rudrapal M, Khairnar SJ, Khan J, Dukhyil AB, Ansari MA, Alomary MN, et al. Dietary polyphenols and their role in oxidative stress-induced human diseases: Insights into protective effects, antioxidant potentials and mechanism (s) of action. *Frontiers in pharmacology*. 2022;13:283.
- 32] Wodehouse RP. Pollen grains. Their structure, identification and significance in science and medicine. Pollen grains Their structure, identification and significance in science and medicine. 1935.
- 33] Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical biochemistry*. 1979;95(2):351-8.
- 34] Young I, Trimble E. Measurement of malondialdehyde in plasma by high performance liquid chromatography with fluorimetric detection. *Annals of clinical biochemistry*. 1991;28(5):504-8.
- 35] Tiwari BK, Pandey KB, Abidi A, Rizvi SI. Markers of oxidative stress during diabetes mellitus. *Journal of biomarkers*. 2013;2013:1-8.
- 36] Papuc C, Goran GV, Predescu CN, Tudoreanu L, Ştefan G. Plant polyphenols mechanisms of action on insulin resistance and against the loss of pancreatic beta cells. *Critical reviews in food science and nutrition*. 2021;62(2):325-52.
- 37] Cheng N, Chen S, Liu X, Zhao H, Cao W. Impact of *schisandrachinensis* bee pollen on nonalcoholic fatty liver disease and gut microbiota in highfat diet induced obese mice. *Nutrients*. 2019;11(2):346.
- 38] Rzepecka-Stojko A, Stojko J, Jasik K, Buszman E. Anti-atherogenic activity of polyphenol-rich extract from bee pollen. *Nutrients*. 2017;9(12):1369.
- 39] Jaradat N, Al-Lahham S, Abualhasan MN, Bakri A, Zaide H, Hammad J, et al. Chemical constituents, antioxidant, cyclooxygenase inhibitor, and cytotoxic activities of *Teucrium pruinosum* boiss. Essential oil. *BioMed Research International*. 2018;2018.
- 40] Zhao W, Xiao Z-J, Zhao S-P. The benefits and risks of statin therapy in ischemic stroke: a review of the literature. *Neurology India*. 2019;67(4):983.
- 41] Bassalat N, Taş S, Jaradat N. *Teucrium leucocladum*: an effective tool for the treatment of hyperglycemia, hyperlipidemia, and oxidative stress in streptozotocin-induced diabetic rats.

